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14. ABSTRACT

Our findings reveal that EMMPRIN immunoreactivity was primarily detected among the glandular epithelial cells; EMMPRIN levels progressively increased with increasing age of TRAMP mice (6-27wks); with the highest detected in liver metastases. Quantitative analysis revealed that by 27-wks (an age exhibiting highly aggressive prostate tumor phenotype), EMMPRIN expression increased significantly (P=0.001). These results suggest that EMMPRIN may have diagnostic value in prostate cancer detection in advanced disease.

15. SUBJECT TERMS
EMMPRIN, prostate cancer, metastasis

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DOD Synergistic Grant Annual Report: W81XWH-08-1-0431 (2010-2011)

(Initiating PI: Haining Zhu)

Partnering-PI: Natasha Kyprianou

Introduction: The focus of this collaborative work has been the identification of EMMPRIN, a membrane protein found to be overexpressed in prostate cancer epithelial cells that exhibit a highly metastatic potential. Previous evidence identified the involvement of EMMPRIN in cancer development and progression via controlling extracellular matrix remodeling and anchor independent growth by stimulating MMP production, angiogenesis via VEGF by activation of AKT-PIK3 pathway, and cell invasion by up-regulation of urokinase-type palsminogen activator. The emerging rationale on pursuing the role of EMMPRIN as a functional biomarker in prostate cancer metastasis led us to determine the status of additional proteins that control the actin-cytoskeleton organization, such as cofilin.

Body: Targeting of tumor cell metastasis is of major therapeutic significance and its exploitation may lead to the identification of effective new modulations such as : (**1**) reversing the ability of tumor cells of becoming resistant to anoikis, therefore making them more susceptible to anoikis-inducing agents; (**2**) interfering with the seeding process of tumor cells into secondary places by making tumor cells non-sensitive to the chemotatic and environmental cues of the new target organ; and (**3**) making these secondary targets less “appealing” to the cancer cells by blocking key molecules promoting cancer cell seeding and survival. Membrane proteins play a critical role during the metastasis process since they regulate cell-cell interactions and coordinate cell-tumorenvironment communication. The initiating PI supported by this PCRP Synergistic Grant (USAMRMC PC074317), Dr. Zhu by utilizing proteomic approaches, identified EMMPRIN as one of differentially expressed membrane proteins in prostate cells, revealing considerably higher levels of EMMPRIN protein in highly metastatic human prostate cancer cells. Subsequent validation studies in Dr. Kyprianou’s lab led to the identification of additional proteins that regulate the cytoskeleton organization as potential regulators of prostate cell migration, cell-cell interactions and ultimately invasion and metastasis.

(1) Ongoing Work: Ongoing studies focus on a) the mechanistic dissection of EMMPRIN’s contribution to metastasis and b) the significance of EMMPRIN in human prostate cancer progression to metastastic disease and clinical outcomes to define

the potential value of this player as a marker of metastasis, studies will pursue expression profiling of EMMPRIN proteein levels in a series of human prostate cancer specimens of increasing Gleason grade and metastastic lesions. Human prostate tissue specimens from patients with primary and metastatic prostate tumors (Department of Pathology, University of Pittsburgh), were subjected to immuno-profiling for EMMPRIN expression and quantitative analysis will be achieved using computer-image analysis in normal prostate; benign prostate hyperplasia, BPH; prostate primary tumors (Gleason Score range 6-9); and metastatic lesions (n=45). Ongoing translational studies focus on establishing a correlation between EMMPRIN expression with serum PSA levels, Gleason grade and patient (disease-free) survival in a large cohort of prostate cancer patients, which may define the value of EMMPRIN as a cancer metastasis marker.

(2) Ongoing experiments investigate the expression of a critical tight junction protein, ZO-1 in prostate tumors with increasing grade. Preliminary staining revealed clear striations of Tight Junctions visualized in epithelial regions that are strongly detected in low-grade tumors and expression is decreased with increasing age of the TRAMP mice. Prostate tumors from 20, 24, 27 and 31-week-old mice are currently being interrogated for tight junction protein expression that will be correlated with the EMMPRIN expression (an inverse correlation is expected). Please see attachment.

(3) Experiments will be pursuing the consequences of EMMPRIN loss/silencing in prostate cancer cells on the transcriptional regulation of the major players of the process of Epithelial Mesenchymal Translation (EMT). The prostate tumor microenvironment represents a key component of the invasive dynamic of prostate cancer. In reference to this new exciting direction of the work supported by this program, please see as Appendices 1 and two papers published by Dr. Kyprianou's group , an original article and a review article [(Zhu and Kyprianou, The FASEB Journal, 24: 769-777, 2010); (Matuszak and Kyprianou, Expert Rev. Endocrinol. Metabolism, 6: 1-14, 2011)] demonstrating the ability of androgens to induce EMT of prostate cancer epithelial cells.

Key Research Accomplishments: In our collaborative interaction we showed that EMMPRIN loss in human prostate cancer cells, had no significant consequences on prostate cell growth, proliferation or apoptosis. We found, however, a significant suppression in prostate tumor cell invasion, migration and metastatic ability using in vitro assays. These data are

reported in the manuscript to be published in the Prostate (please see as Appendix 3 , a copy of proof-print of the manuscript by Zhu et al, 2011) .

My contributions at the translational level have been the determination of the potential predictive value of EMMPRIN in prostate cancer progression, first utilizing the TRAMP mouse model of prostate tumorigenesis and subsequently analyzing a series of human prostate cancer specimens of increasing Gleason grade. The TRAMP mice (C57BL/6J) are transgenic mice that express SV40T/t antigens under the prostate specific rat probasin promoter. TRAMP transgenic males develop prostate adenocarcinoma in a manner resembling the clinical progression of human prostate cancer from intra-epithelial neoplasia to androgen-independent metastatic tumors. Hematoxylin and eosin (H&E)-stained sections of prostate tissues from TRAMP/+/+ male mice were evaluated (by N.K.) to confirm pathological grade. Prostate sections from wild type and the TRAMP tumors of increasing grade and metastatic lesions (5μm), were subjected to immunohistochemical analysis for EMMPRIN expression. Slides are examined under a fluorescence microscope and expression is determined in a semiquantitative fashion, incorporating both the staining intensity and the number of positively stained epithelial cells. As shown in Figure 1 histopathological grading of prostatic tumors revealed that in the majority of 16wk-24wk-old TRAMP mice, prostate adenocarcinoma was evident (16-20weeks),and with increasing age (24 weeks), poorly differentiated tumor foci were detected with focal cribriform lesions protruding into the lumen (grade 3-5), representing tumor progression to advanced disease. A score for each histological grade (H) was determined as the product of intensity and proportion ($H = I \times P$).

Reportable Outcomes: The expression profile of EMMPRIN expression was assessed during *in vivo* prostate tumorigenesis in the TRAMP model of prostate cancer progression is shown on Figure 1. Our findings reveal strong EMMPRIN immunoreactivity primarily detected among the glandular epithelial cells of prostate tumors from TRAMP mice. Quantitative analysis of the immunoreactivity profile for EMMPRIN revealed that by 24-wks (an age exhibiting highly aggressive prostate tumor phenotype), EMMPRIN expression increased significantly ($P=0.001$). These results suggest that EMMPRIN may have diagnostic value in prostate cancer detection in advanced disease.

Preliminary analysis of the expression of EMMPRIN in human specimens revealed the low immunoreactivity in premalignant lesions of the prostate, i.e. high grade Prostatic Intraepithelial Neoplasia (Figure 2, panel A). The significance of the tumor microenvironment, as dictated by EMMPRIN in promoting the metastatic spread, is highlighted in the immunostaining

profile among the epithelial benign areas of a human prostate tissue, adjacent to tumor foci that are totally negative for EMMPRIN (Figure 2, Panel B).

Moreover, the expression of cofilin and phosphorylated cofilin, correlated with the profiling of the prostate tumor invasion.

Figure 1

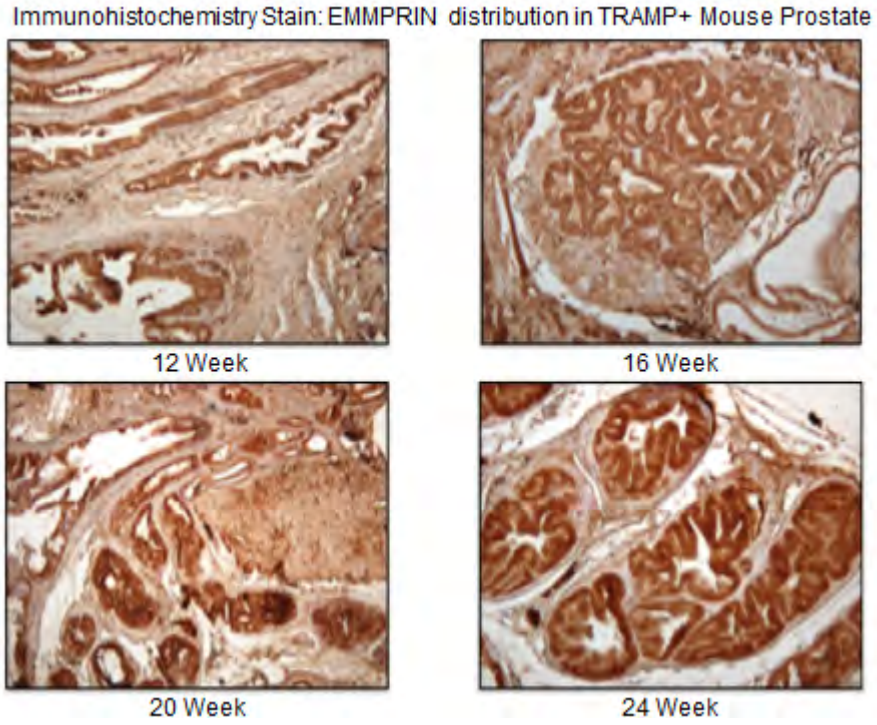
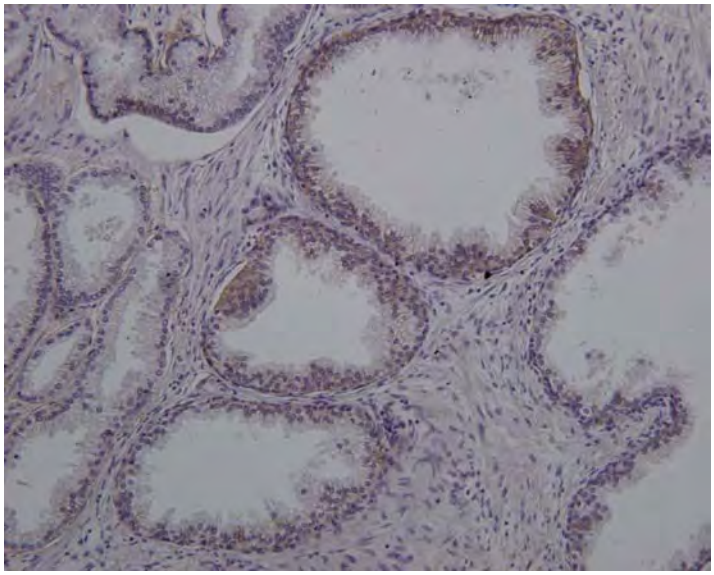
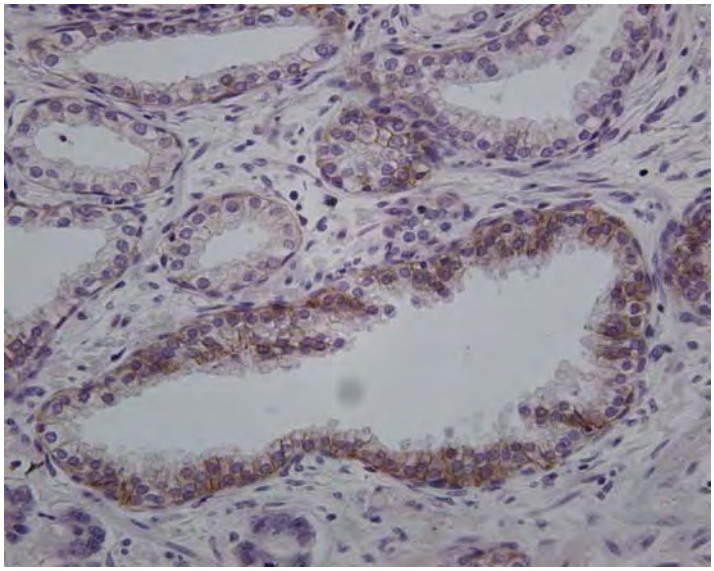


Figure 2



Panel A: Weak EMMPRIN immunoreactivity is detected in areas of High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) in human prostate cancer.



Panel B: Significance of prostate tumor microenvironment. Strong EMMPRIN expression among prostate benign epithelial cells adjacent to tumor glands (negative).

Figure 3A

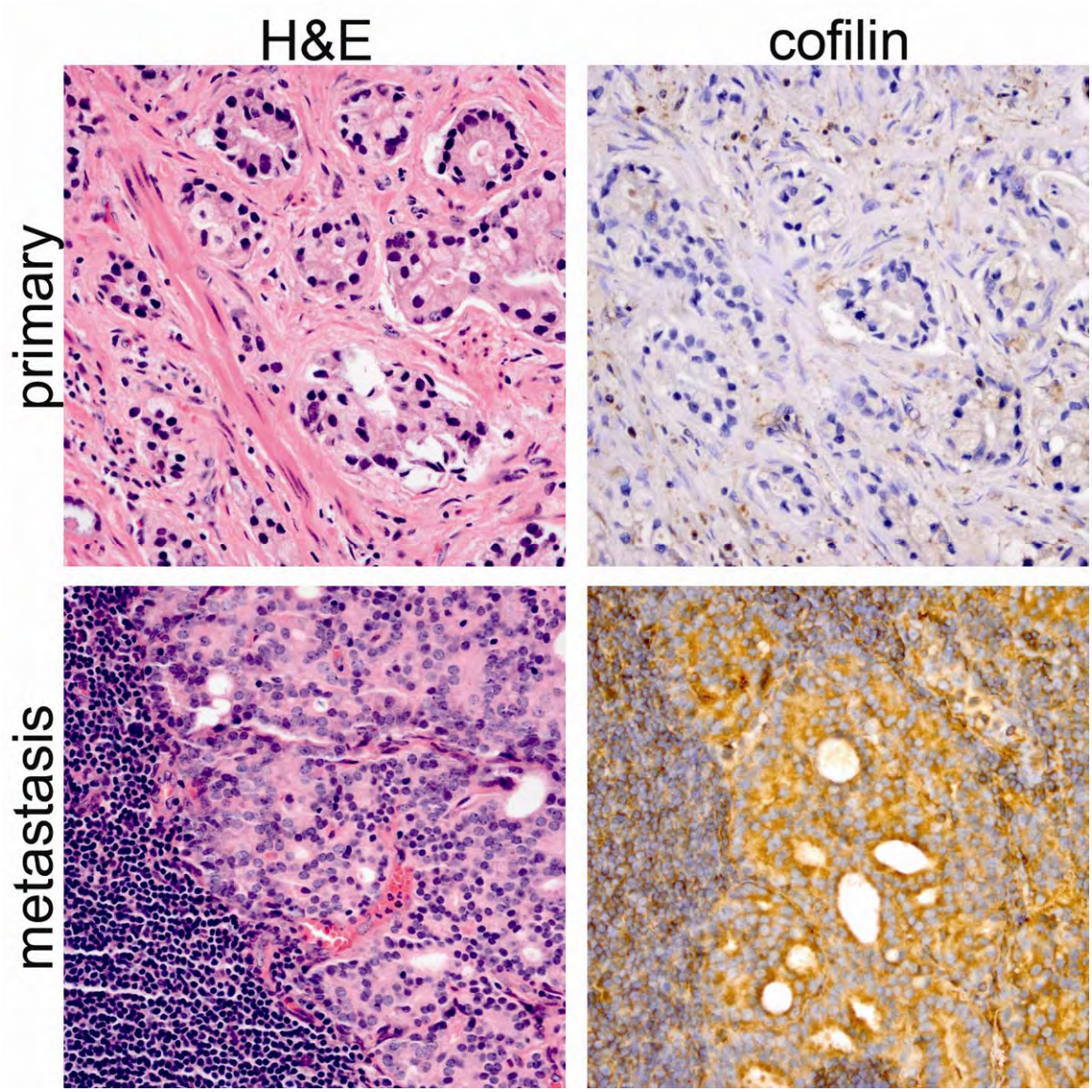
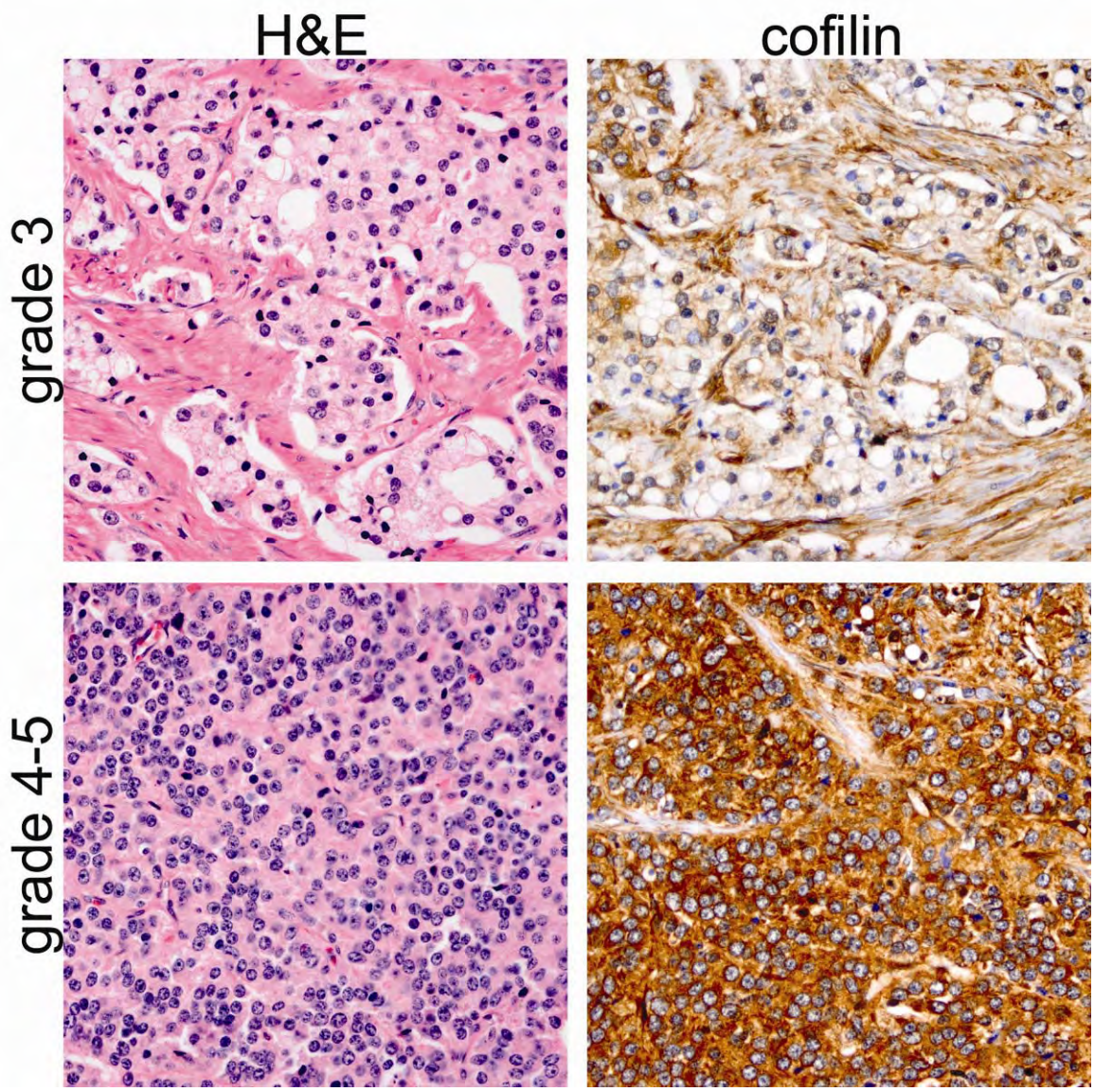


Figure 3B



Publications Resulting from last year's funding support:

Desiniotis, A. and **Kyprianou, N.** Significance of Talin in Cancer Progression and Metastasis, *International Review of Cell and Molecular Biology,* Elsevier, 2011; Ch. 4, Volume 289, pp.117-147.

Desiniotis, A. and **Kyprianou, N**. Advances in the Design and Synthesis of Prazosin Derivatives over the Last Ten Years. *Expert Opinion in Therapeutic Targets*, 15(12):1405-1418, 2011.

Tang, X., Tang, X., Gal, J., **Kyprianou, N.,** Zhu, H. and Tang, G. Detection of microRNAs in prostate cancer cells by microRNA array. *Methods in Molecular Biology: MicroRNAs in Development,* 732:69-88, 2011.